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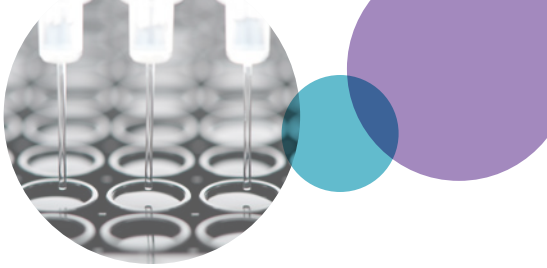

# Super Streptavidin (SSA) Biosensors

*Label-free analysis of small molecule-protein interactions*

## Key features

- Enables immobilization of any protein in high density via streptavidin
- Designed for use in small-molecule and fragment screening and kinetic characterization

## Overview

Small molecule kinetics can be rapidly measured in high throughput on the Octet® RED and RED384 instruments. In a typical experiment, a biotinylated protein target is immobilized onto a high-capacity Super Streptavidin (SSA) Biosensor surface, and this surface is exposed to a solution of the small molecule in a microplate well. The association of the small molecule to the target protein on the biosensor is measured over time. Finally, the biosensor is moved to a well containing buffer to monitor the dissociation of the small molecule from the target protein. Rate constants can be calculated from the binding data, including on-rate ($k_{on}$ or $k_a$), off-rate ($k_{off}$ or $k_d$), and equilibrium dissociation constant ($K_D$).

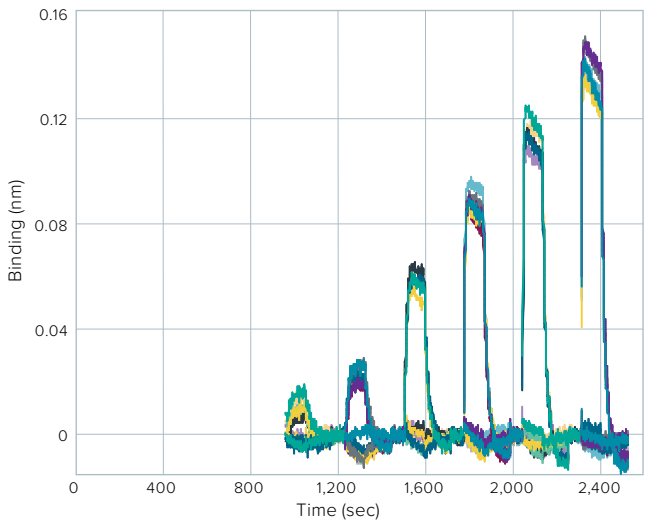


Figure 1: Processed data for the binding of furosemide (330 Da) to carbonic anhydrase. Data shown is a result of the subtraction of the biocytin reference biosensor data from the carbonic anhydrase biosensor data. The binding of furosemide at 0.12, 0.37, 1.1, 3, 10, and 30 µM is clearly visible.

## Quick facts

- Immobilization Chemistry: High-density streptavidin
- Baseline Stability: 60 minutes
- Number of Acceptable Regeneration Cycles: protein dependent

## Use of Super Streptavidin biosensors

When performing small molecule analysis on the Octet RED or RED384 system, each SSA Biosensor can be used for multiple analyses since most small molecules have affinities greater than 1 nM and thus dissociate fully after several minutes. Typical experiments include both biosensors immobilized with the biotinylated target protein (target biosensors) and biosensors blocked with biocytin (reference biosensors). Figure 1 shows data for the binding of furosemide (330 Daltons) to carbonic anhydrase collected on an Octet RED system. The precision for a typical run on the Octet RED system is shown in Table 1.

## Data analysis

Analysis of small molecule kinetic data is easy using Octet Data Analysis software. The software supports both Global analysis and Steady State analysis of kinetic data sets. Global analysis derives a single set of parameters including $R_{max}$, $k_{on}$, $k_{off}$, and $K_D$ from a set of association and dissociation curves from a concentration series (see the *Octet Data Analysis User Guide* for more information). This method generates more precise and accurate data than results obtained from association and dissociation data from a single concentration.

An example of global fitting of a titration series is shown in Figure 2. The $K_D$ can also be derived from equilibrium responses using Steady State analysis. This method does not generate $k_{on}$ and $k_{off}$ values. The experiment requires a concentration-dependent response, and dilutions of 2–4X are recommended for a six-point concentration series (minimum). An example is shown in Figure 2 (right).

| Analysis | $R_{max}$ (Δnm) | $R_{max}$ Error | $k_{off}$ (1/s) | $k_{on}$ (1/Ms) | $k_{on}$ Error | $K_D$ (M) | Chi² | R² |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.0975 | 0.0001 | 7.83E-02 | 6.49E+04 | 4.57E+02 | 1.21E-06 | 0.053 | 0.99 |
| 2 | 0.1017 | 0.0001 | 7.88E-02 | 5.75E+04 | 3.84E+02 | 1.37E-06 | 0.050 | 0.99 |
| 3 | 0.0951 | 0.0002 | 8.52E-02 | 6.76E+04 | 5.66E+02 | 1.26E-06 | 0.067 | 0.99 |
| 4 | 0.0976 | 0.0002 | 7.97E-02 | 6.20E+04 | 4.69E+02 | 1.28E-06 | 0.059 | 0.99 |
| 5 | 0.0931 | 0.0001 | 8.32E-02 | 8.72E+04 | 6.97E+02 | 9.54E-07 | 0.063 | 0.99 |
| Avg | 0.097 | 0.0002 | 0.081 | 67836 | 515 | 1.22E-06 | 0.058 | 0.989 |
| SD | 0.003 | 0.0002 | 0.003 | 11453 | 121 | 1.57E-07 | 0.007 | 0.002 |

Table 1: Precision of furosemide analysis with carbonic anhydrase on the Octet RED system.

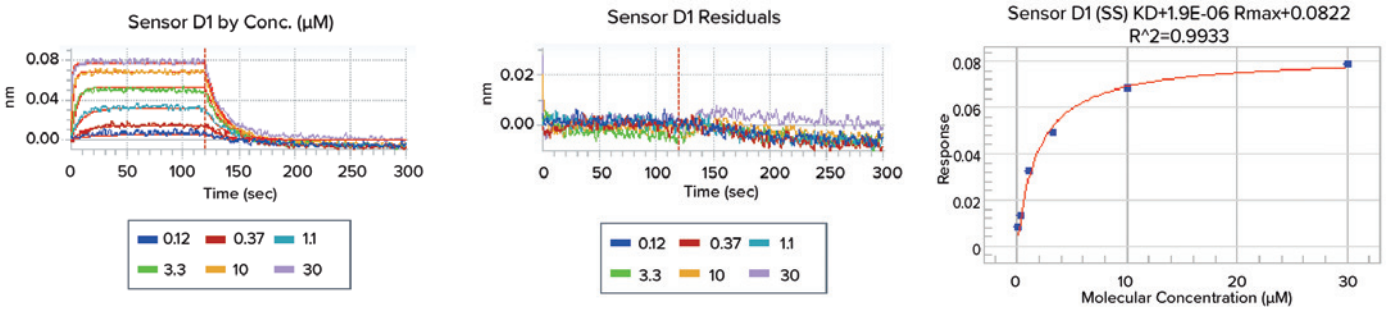


Figure 2: Global analysis of small molecule binding data at 0.12–30 µM (left), residuals for curve fitting (center), and the steady-state plot (right).

Dip and Read™ Super Stretavidin biosensors are designed for use with the most senstive Octet instruments, the Octet RED and RED384 systems. The latest version of software includes a predefined protocol to make this assay easy to run and is available with 21 CFR Part 11 compliance tools.

*For more information about the Octet and BLItz platforms for label-free, real-time detection of biomolecular interactions, applications, and services, visit www.fortebio.com or contact us directly.*

## Ordering information

| Part no. | UOM | Description |
|---|---|---|
| 18-5057 | Tray | One tray of 96 Super Streptavidin biosensors coated with streptavidin for small molecule kinetic analysis |
| 18-5065 | Pack | Five trays of 96 Super Streptavidin biosensors coated with streptavidin for small molecule kinetic analysis |
| 18-5070 | Case | Twenty trays of 96 Super Streptavidin biosensors coated with streptavidin for small molecule kinetic analysis |

Note: Additional materials are required to run these assays. Please consult Technical Note 16 for full details.

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